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Research Article

STATISTICAL OPTIMIZATION OF BUDESONIDE PELLETS COATED WITH EUDRAGIT RLPO POLYMER FOR POSSIBLECOLONIC DRUG DELIVERY

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ABSTRACT

Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity and weak mineral corticoid activity. Corticosteroids have been shown to have a wide range of inhibitory activities against multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, and lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, and cytokines) involved in allergic and non-allergic-mediated inflammation. These anti-inflammatory actions of corticosteroids may contribute to their efficacy in ulcerative colitis, crohn's disease and inflammatory bowel disease. Present study was carried out to investigate the colon specific delivery of the Budesonide by using eudragit RLPO. The effect of different processing variables of fluidized bed processor like fluidization pressure, atomization pressure and feed rate on the quality of drug layering and polymer coating were studied. The Box-Behnken experimental design applied for optimization of processing parameters of fluidized bed processor. All trials of drug layering were done and for each trial the drug content was determined. The results of drug layering reveal that as the fluidization pressure was increased, the drug content was also increased. As atomization pressure and feed rate increases, the drug content decreases. For each trial of polymer coating, the weight gain and drug release was determined. The results of polymer coating reveals that as the fluidization pressure and feed rate increases, the weight gain decreases and drug release increases. But when atomization pressure increases, the weight gain increases, and drug release decreases. The processing variables given by design expert software for drug layering of optimized batch was fluidization pressure 0.37, atomization pressure 0.52 and feed rate 1.75 rpm. The processing variables given by design expert software for polymer coating of optimized batch was fluidization pressure 0.40, atomization pressure 0.50 and feed rate 2 rpm.

Keywords: Budesonide, colon specific drug delivery, Eudragit RLPO.

INTRODUCTION

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amoebiasis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs. [1,2] The colon specific drug delivery system (CDDS) should be capable of protecting the drug en route to the colon i.e. drug release and absorption should not occur in the stomach as well as the small intestine, and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon.[3] The colon is thought to be the most significant absorption site for peptides and protein drugs because of limited diversity and intensity of digestive enzymes, beside this proteolytictivity shown by the colon is much lesser than that of the small intestine. CDDS protects peptide drugs from hydrolysis, and enzymatic degradation in duodenum and jejunum, and eventually releases the drug into ileum or colon which leads to greater systemic bioavailability [4] and finally, because the colon has a long residence time which is up to 5 days and is highly responsive to absorption enhancers. [5] Corticosteroids such as hydrocortisone and prednisolone are administered via the rectum for the treatment of ulcerative colitis. [6-8]

Budesonide is a glucocorticoid steroid for the treatment of asthma and non-infectious rhinitis (including hay fever and other allergies), and for treatment and prevention of nasal polyposis. In addition, it is used for Crohn's disease (inflammatory bowel disease). Budesonide has a high first-pass metabolism. It has efficacy in the terminal ileum and the right colon. Budesonide in comparison with prednisolone has been associated with fewer bone density losses, and, unlike other corticosteroids, has little influence on the hypothalamic-pituitary-adrenal axis, which also limits the need of tapering before discontinuation. Overall, it has a lower incidence of systemic manifestations than similar medications.[9,10]

MATERIALS AND METHODS

Budesonide was obtained as gift sample from Glenmark Pharmaceuticals Ltd., Nasik. EudragitL100 and EudragitS100 were obtained as gift sample from Colorcon Ltd., Goa. Eudragit RLPO and Eudragit RSPO were obtained as gift sample from Lupin Pharmaceuticals, Pune. Ethyl Cellulose was obtained as gift sample Alkem Pharmaceuticals Ltd., Mumbai. Other materials used were of AR Grade and were purchased from Modern Scientifics, Nashik.

Drug-excipients compatibility study

Physical observation

A compatibility study was carried out with potential formulation excipients to determine drug- excipients interaction. All the physical mixtures of model drug and excipients in 1:1 ratio were kept under compatibility study for 14 days at 55°C in glass vials sealed and were observed physically for caking, liquefaction, discoloration and odour or gas formation.

Differential Scanning Calorimetry

Drug-excipient interaction study was done by using DSC. In this study, thermogram of pure drug, mixtures of drug: excipient was taken. The drug, the drug polymer mixture (1:1), mixture of polymers (1:1) were kept in the dried glass vial under normal conditions at room temperature for 7 days; these samples were analyzed for Differential scanning calorimetry (DSC) using Shimadzu DSC-50 instrument equipped with a computerized data station. Samples (3–4 mg) were placed in an aluminium pan and heated at a rate of 10°C/min with air in the reference pan in an atmosphere of nitrogen to a temperature of 250°C.

Preliminary Trials

Drug Loading [11]

Pellets (100 g, mesh no. 25-30) were used as initial cores to achieve drug loading. Budesonide was incorporated on non-pareils seeds by spraying Budesonide solution in ethanol containing polyvinyl pyrrolidone (PVP 30K) as a binder and talc as antisticking agent. The coating solution was sprayed over the non-pareils seed by using Fluidized bed processor. Composition for first trial of Drug layering was given in table no.1. A laboratory size Miniglatt fluidized bed coating machine was used for coating the drug solution. The flow rate was maintained constant such that no agglomeration of the beads occurred during the coating process. The air flow was kept intermediate level to achieve good drying efficiency. During the

layering process, the beads were intermittently dried for 10 min at room temperature. After layering, the beads were collected. Using the composition as shown in Table 1 for drug layering solution, different trials (Table 2) were taken to select the processing variables.

Table 1: Composition for first trial of Drug layering

Sr. No.	Ingredients	Qty in gms
1	Sugar spheres (25/30)	10
2	Budesonide	1
3	PVP30K	0.05
4	Ethanol	5
5	Talc	0.05

Table 2: Trials for the selection of Process Parameters for drug solution layering on sugar sphere.

Sr.No.	Process Parameters	Conditions				
		R1	R2	R3	R4	R5
1	Sugar spheres (gm)	100	100	100	100	100
2	Spray rate (rpm)	0.80	1.10	1.50	1.80	2.00
3	Atomizing air pressure (bar)	0.3	0.4	0.5	0.6	0.7
4	Product temperature (^{0}c)	30-35	35-40	40-45	35-40	37-43
5	Inlet temperature (^{0}c)	45	50	55	50	50
6	Fluidizing pressure (bar)	0.2	0.3	0.35	0.4	0.45

Selection of Drug Concentration for Drug Layering

Then the preliminary studies for various combinations as shown in Table 3 were tried keeping the process parameters constant as shown in Table 2 to achieve drug content. On the basis of the trial T1 to T5 the composition for the drug layering was selected as shown in Table 3. The composition for the drug layering solution was selected from the evaluation data.

Table 3: Trial using different composition of drug and excipients

Sr. No.	Ingredients	Batch No (Qty in gms)				
		T1	T2	T3	T4	T5
1	Sugar spheres (25/30)	10	10	10	10	10
2	Budesonide	1	1.2	1.4	1.4	1.4
3	PVP30K	0.05	0.05	0.1	0.1	0.1
4	Ethanol	5	5	5	5	5
5	Talc	0.05	0.05	0.05	0.1	0.1

Evaluation of the drug layered pellets

Drug Content

Accurately weighed samples of the coated pellets (1gm) from all the formulations were placed in 10 ml ethanol for 10 min then water was added to make 100 ml. The dissolved pellets were filtered and analyzed spectrophotometrically for Budesonide content at 247 nm. A calibration curve was used based on standard solutions in ethanol water. The other excipients used in the coating did not interfere with the analysis at this wavelength. All experiments were performed in triplicate.

Polymer coating

Preparation of Polymer Coating Solution

The composition shown in Table 9 was used for the preperation of polymer solution. The Eudragit RLPO powder was slowly added into 50% of the diluent mixture and stirred until the polymer was completely dissolved. The talc and triethyl citrate was added in the remaining diluent mixture and stirred for 10 min. Excipient suspension was pour slowly into the Eudragit solution with continuous stirring. Composition for Polymer coating solution was given in table 4. The polymer coating solution was prepared. The polymer coating was done using preliminary processing variables.

Table 4: Composition for Polymer coating solution

Sr. No.	Ingredients	Qty based on dry polymer (%)	Qty to be weighed (gm)
1	Eudragit RLPO		0.5
2	Talc	5	0.2
3	Triethyl Citrate	1	0.05
4	Acetone		3.429
5	Isopropyl alcohol		5.2
6	Purified water		0.5

Method for Polymer Coating [12]

Pellets (100 g) which were earlier coated with drug were used for polymer coating. Eudragit RLPO was mixed with solvent system prepared for the preparation of polymer coating solution. Triethyl Citrate was used as the plasticizers. Talc was added to the above dispersion as an anti-adherent to prevent particulate aggregation during the coating process. A laboratory size Miniglatt fluidized bed coating machine was used for coating polymer. The processing parameters were maintained such that proper coating and no agglomeration of the beads occurred during the coating process. During the polymer coating process, the beads were intermittently dried for 10 min at room temperature. After layering, the beads were collected.

Selection of Processing Variables for Polymer Coating

The different trials which are given in Table 5 for polymer coating were conducted. From these trials the preliminary processing variables for polymer coating was selected.

Table 5: Trials for the selection of parameters for polymer coating solution on sugar sphere

Sr. No.	Process Parameters	Conditions				
		G1	G2	G3	G4	G5
1	Spray rate(rpm)	0.50	0.50	0.50	1.00	1.50
2	Atomizing air pressure(bar)	0.3	0.4	0.5	0.6	0.7
3	Product temperature(^{0}c)	20-25	25-27	25-29	25-30	25-30
4	Inlet temperature(^{0}c)	30	40	45	45	45
5	Fluidizing pressure(bar)	0.2	0.3	0.4	0.5	0.55

Selection of Concentration of Polymer for the Polymer Coating

The composition of the polymer coating solution should be selected such that the drug release will be within 12 hr. For that purpose the different batches were designed as given in Table 6. From the obtained data for the polymer coating batches the composition for the polymer coating was selected.

Table 6: Different composition for polymer coating solution

Sr. No.	Ingredients	Batch No (Qty in gms)			
		L1	L2	L3	L4
1	Drug Layered Pellets	5	5	5	5
2	Eudragit RLPO	0.5	0.7	0.8	1
3	Talc	0.25	0.35	0.4	0.5
4	Triethyl Citrate	0.05	0.07	0.08	0.1
5	Acetone	3.5	3.5	3.5	3.5
6	Isopropyl alcohol	5	5	5	5
7	Purified water	0.5	0.5	0.5	0.5

Evaluation of polymer coated pellets

Dissolution studies [13]

Coated pellets (1 gm) were used for determining the in-vitro release of drug. The USP I Basket apparatus was used with 900 ml of Gastric fluid (pH 1.2) for 2 h. After 2 h the dissolution media was changed i.e. Intestinal Fluid (pH 6.8), this is for 3 h. Then after that, change the dissolution medium to phosphate buffer (pH 7.4) at 37 ^{0}C and 50 rpm. Samples (5 ml) were withdrawn at 1, 2, 3, 4, 5, 6, 8, 10, 11 and

12 h and were assayed spectrophotometrically at 247 nm. From the absorbance values, the percent cumulative release of Budesonide was calculated. All the experiments were performed in triplicate. For all the formulations, the measured response selected was cumulative percent dissolved in 12 h.

Experimental design applied for optimization of processing parameters for fluidized bed processor [14-17]

Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery devices. Based on the principle of design of experiments (DoE), the methodology encompasses the use of various types of experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain to determine the optimum processing variables. The technique requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating dosage forms. Box-Behnken design was used to statistically optimize the formulation parameters and evaluate the main effects, interaction effects and quadratic effects of the process parameters of fluidized bed processor on the drug layering and polymer coating. A 3-factor, 3-level design was used to explore the quadratic and linear response surfaces using Design Expert (Version 8.0.1, Stat-Ease Inc., and Minneapolis, MN). Statistical validity of the polynomials was established on the basis of analysis of variance (ANOVA) provision in the Design Expert software. Level of significance was considered at $p < 0.05$. The best-fitting mathematical model was selected based on the comparison of several statistical parameters, including the coefficient of variation (CV), the multiple correlation coefficient(R^2), the adjusted multiple correlation coefficient (adjusted R^2), and the predicted residual sum of squares (PRESS), provided by the software. PRESS indicates how well the model fits the data, and for the chosen model, it should be small relative to the other models under consideration. The 3-D response surface graphs and the 2-D contour plots were also generated by the Design Expert software. These plots are very useful to see interaction effects of the factors on responses.

Experimental design was done for-

1. Optimization of Drug Layering

2. Optimization of Polymer Coating

Box-Behnken design was applied in present study by considering fluidization pressure, atomization pressure and feed rate as independent variables and drug content, weight gain and perecent drug release as dependent variables as shown in Table 12.

Drug Layering

The Table 7 shows the dfferent variabes and its levels used in the optimization design. These variables were used for the drug layering on the sugar spheres. Using these variables at different three levels the trials were designed.

Table 7: Variables in Box-Behnken design, Factor Levels used, Actual (coded) for drug layering

Independent Variables Constrains	Low (-1)	Medium (0)	High (+1)
X1 = Fluidization pressure(bar)	0.10	0.20	0.30
X2=Atomization Pressure (bar)	0.50	0.70	0.90
X3 = Feed Rate (RPM)	2	4	6
Dependent variables Constraints			
Y = Drug content			

Evaluation study for response factor

Drug Content

Accurately weighed samples of the coated pellets (1gm) from all the formulations were placed in 10 ml ethanol for 10 min then water was added to make 100 ml. The dissolved pellets were filtered and analyzed spectrophotometrically for Budesonide content at 247 nm. A calibration curve was used based on standard solutions in ethanol water. The other excipients used in the coating did not interfere with the analysis at this wavelength. All experiments were performed in triplicate.

Polymer Coating

The Table 8 shows the dfferent variabes at three levels used in the optimization design. This variables was used for the polymer coating on the drug layered sugar spheres. Using this variables and its level the trials were designed.

Table 8: Variables in Box-Behnken design, Factor Levels used, Actual (coded) for polymer coating

Dependent Variables Constrains	Low (-1)	Medium (0)	High (+1)
X1 = Fluidization pressure(bar)	0.30	0.40	0.50
X2=Atomization Pressure (bar)	0.40	0.50	0.60
X3 = Feed Rate (RPM)	1	2	3
Independent variables Constraints			
Y_1 = Weight Gain		Y_2= % Cumulative Drug Release	

Evaluation study for response factors

Weight Gain

Weight gain for the polymer coated pellets was determined by

Weight Gain = Final weight – Initial weight

Dissolution studies

Dissolution study was made as stated above for coated pellets by using polymer coated pellets.

Evaluation optimized processing variables batch

Scanning electron microscopy (SEM)

Scanning electron photomicrographs of pellets were taken. To understand changes in the surface morphology, the topography of pellets was analyzed with help of scanning electron microscopy. A small amount of pellets was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 20 kV, chamber pressure of 0.6 mm Hg, with original magnification 27X. Pellet surfaces were evaluated before and after coating.

RESULTS AND DISSCUSSION

Drug-excipients compatibility study

Physical observations

Significant physical change was not observed for Budesonide and inactive excipients those were kept under compatibility study for 14 days at 55 ^{0}C in glass vials sealed showed that the active and inactive ingredients compatible with each other.

Differential Scanning Calorimetry

The DSC studies revealed that budesonide has prominent, characteristic endothermic peak at 255 ^{0}C. This endothermic peak signifies that Budesonide used as in pure state. Physical mixture of budesonide: Ethyl cellulose: Eudragit RLPO (1:1:1) shows no deviation of endothermic peak of budesonide. This revealed that there was no chemical reaction between drug and the polymers used.

Evaluation of preliminary trials

Selection of Processing Variables for Drug Layering

The different trials were taken as mention in Table 2 for the selection of different processing variables of fluidized bed processor. In R1 and R2 trials after spraying of drug layering solution there were agglomeration of the pellets was observed due to high spray

rate and low inlet temperature or low atomization pressure. The R5 trail processing variables was taken into consideration for determination of drug layering (Table 9).

Table 9: Parameters for drug solution layering of sugar sphere

Sr. No.	Process Parameters	Conditions
1	Spray rate	1-2rpm
2	Atomizing air pressure	0.3-0.5 bar
3	Product temperature	35-40^{0}c
4	Inlet temperature	45-50^{0}c
5	Fluidizing pressure	0.2-0.3 bar

Selection of Drug Concentration for Drug Layering

Evaluation of the drug layered pellets

The trials are taken using initial composition shown in Table 1 having drug content upto 50%. For the improvement of the drug content the different trials were designed. Drug content of preliminary trial was given in table 10. The different composition of the material was taken as shown in the Table 3. From all preliminary trials the T5 combination was selected as in this trial drug content was achieved.

Table 10: Drug content of preliminary trial

Sr. No.	Trial No.	Drug Content (mg/gm)
1	T1	32.69±1.6
2	T2	47.40±1.2
3	T3	66.48±0.3
4	T4	98.55±0.7
5	T5	101.25±0.9

Selection of Processing Variables for Polymer Coating

Using the selected processing variable the polymer coating on the drug layered pellets was done and the drug release from that coated pellets was determined. The Table 11 shows the selected parameter range for polymer coating.

Table 11: Parameters range for polymer coating on drug coated pellets

Sr. No.	Process Parameters	Conditions
1	Spray rate	1-2 rpm
2	Atomizing air pressure	0.50-0.70bar
3	Product temperature	26-30^{0}c
4	Inlet temperature	40-45^{0}c
5	Fluidizing pressure	0.40-0.55bar

Evaluation of polymer coated pellets

Dissolution studies

The different composition was chosen to achieve the drug release in 12hr. Results were mention in Table 12. Using the selected processing variables the polymer coating on the drug layered pellets was done. The study of drug release was done after polymer coating. In the L1 trial the drug release in 2 h at 1.2 pH. In the polymer coating the polymer used was enteric polymer but the drug release at 1.2 pH. Then concentration of polymer was increased. In the L3 trial, 88.45 % of the drug was released in 8 h in 7.4 pH. Then the polymer concentration was further increased. In the L4 trial the drug was released in 12 h in 7.4 pH. The composition of the last trial was selected for the polymer coating.

Table 12: Evaluation of polymer coated pellets for drug release.

Dissolution Fluid	Time	% Drug Release			
	(h)	L1	L2	L3	L4
Gastric Fluid (1.2)	1	65.24	18.23	1.23	0.164
	2	87.55	50.20	4.16	2.52
Intestinal Fluid	3		56.96	12.20	3.93
(6.8)	4		65.23	29.02	5.83
	5		85.13	40.60	12.68
Phosphate Buffer	6			50.12	28.28
(7.4)	7			73.92	46.67
	8			88.45	63.92
	9			79.01	
	10			85.93	
	11			85.99	
	12			86.01	

Optimisation data analysis

Experimental design

Use of experimental design allows for testing a large number of factors simultaneously and precludes the use of a huge number of independent runs when the traditional step-by-step approach is used. Systematic optimization procedures are carried out by selecting an objective function, finding the most important or contributing factors and investigating the relationship between responses and factors by the so-called response surface methodology. Objective function for the present study was selected as maximizing the drug layering and polymer coating efficiency while studying its effect on drug content and drug release.

Box-Behnken design was used to statistically optimize the processing parameters and evaluate the main effects, interaction effects and quadratic effects of the processing parameters on the drug layering and coating efficiency of enteric coated formulation. A 3-factor, 3-level design was used to explore the quadratic response surfaces and for constructing second order polynomial models using Design Expert (Version 8.0.0, Stat-Ease Inc., Minneapolis, MN). The Box-Behnken design was specifically selected since it requires fewer runs than a central composite design, in cases of three or four variables. This cubic design is characterized by set of points lying at the midpoint of each edge of a multidimensional cube and center point replicates (n = 3) whereas the 'missing corners' help the experimenter to avoid the combined factor extremes. This property prevents a potential loss of data in those cases. A design matrix comprising of 17 experimental runs was constructed, for which the linear computer generated quadratic model is defined as;

$$Y=b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where, Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y from experimental runs; and X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_1X_2 and X_{2i} (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively.

Independent variables studied were fluidization pressure (X_1), atomization pressure (X_2) and the feed rate (X_3). The dependent variable was the drug content and Weight Gain and % Cumulative drug release (Y). The range of independent variables under study is shown in Table 12 & 13 along with their low, medium and high levels, which were selected based on the results from preliminary experimentation. The fluidization pressure (X_1), atomization pressure (X_2) and feed rate (X_3) used to prepare the 17 experimental formulations.

The polynomial equations can be used to draw conclusion after considering the magnitude coefficient and the mathematical sign that the coefficient carries. A high positive or negative value in the equation represent that by making a minor change in the setting of that factor one may obtain a significant change in the dependent variable.

Statistical validity of the polynomials was established on the basis of analysis of variance (ANOVA) provision in the Design Expert software. Level of significance was considered at > F less than 0.05. The best-fitting mathematical model was selected based on the comparison of several statistical parameters, including the coefficient of variation (CV), the multiple correlation coefficient(R^2), the adjusted multiple correlation coefficient (adjusted R^2), and the predicted residual sum of squares (PRESS), provided by the software. PRESS indicates how well the model fits the data, and for the chosen model, it should be small relative to the other models under consideration. The 3-D response surface graphs and the 2-D contour plots were also generated by the Design Expert® software. These plots are very useful to see interaction effects of the factors on

responses. Actual Layout of Design for Drug Layering was given in table 13

Table 13: Actual Layout of Design for Drug Layering.

Run	Factor 1 Fluidization (bar)	Factor 2 Atomization (bar)	Factor 3 Feed Rate (rpm)	Response %Drug Content
D 1	0.20	0.50	2.00	101.34±1.1
D 2	0.20	0.70	4.00	63.56±2.9
D 3	0.20	0.70	4.00	64.5±2.4
D 4	0.30	0.70	2.00	82.16±1.6
D 5	0.10	0.90	4.00	12.27±0.5
D 6	0.20	0.50	6.00	5.65±0.5
D 7	0.30	0.70	6.00	9.57±0.1
D 8	0.10	0.50	4.00	68.45±0.78
D 9	0.20	0.70	4.00	68.43±2.7
D 10	0.10	0.70	2.00	80.26±1.7
D 11	0.20	0.70	4.00	64.65±1.4
D 12	0.30	0.50	4.00	94.55±1.7
D 13	0.10	0.70	6.00	7.67±0.23
D 14	0.30	0.90	4.00	92.86±1.7
D 15	0.20	0.90	6.00	13.23±0.5
D 16	0.20	0.70	4.00	66.76±1.01
D 17	0.20	0.90	2.00	87.08±0.98

Drug Layering

The Table 13 shows the different trials design by using optimization design and response obtained after trial was run.

Full and Reduced Model assessment for the dependent variables

The ranges of responses Y was 5.65-101.34%. All the responses observed for seventeen processing variables were fitted to various models using Design- Expert software. It was observed that the best-fitted models were linear. The values of R^2, adjusted R^2, predicted R^2, SD and %CV are given in Table 14, along with the regression equation generated for each response. It was observed that the independent variable viz. X_1 (Fluidization pressure) had a positive effect on drug content (Y). Another independent variables viz. X_2 (Atomization Pressure) and X_3 (Feed Rate) had a negative effect (Y).

Table 14: Summary of results of regression analysis for responses Y.

Models	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response (Y) Linear model	0.7807	0.7301	0.5575	17.66	30.54

Model equation

Drug content = +57.82 + 13.81 * X_1 - 8.07 * X_2 - 39.34 * X_3

Statistical validation of the polynomial equations generated by Design Expert and estimation of significance of the models was established on the basis of analysis of variance provision of the software as shown in Table 15. The Model F-value of 15.43 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In Table 15, p values for response Y (Drug content) represent that the linear contribution (X_1 & X_3) is significant model term and the linear contribution (X_2) is non-significant model term. The values obtained for main effects of the independent variables from Equation indicate that fluidization pressure (X_1) has positive effect on the response Y (Drug content). From the Figure 1 and 2 of the response curve of drug content for drug coated pellets, it is observed that as the fluidization pressure increases from -1 level (0.10 bar) to 0(0.20 bar) and +1 level(30 bar), drug content of drug layered pellets increases significantly. From equation indicate that atomization pressure (X_2) has negative effect on the response Y (Drug content).On the other hand, the atomization pressure increases from -1 level (0.50 bar) to 0 level (0.70 bar) and +1 level (90 bar), drug content of drug layered pellets was decreased. The equation indicates that feed rate (X_3) has five times negative effect than that of the atomization pressure on the response Y (Drug content). The feed rate increases from -1 level (2 RPM) to 0 level (4 RPM) and +1 level (6 RPM), drug content of drug layered pellets was decreased.

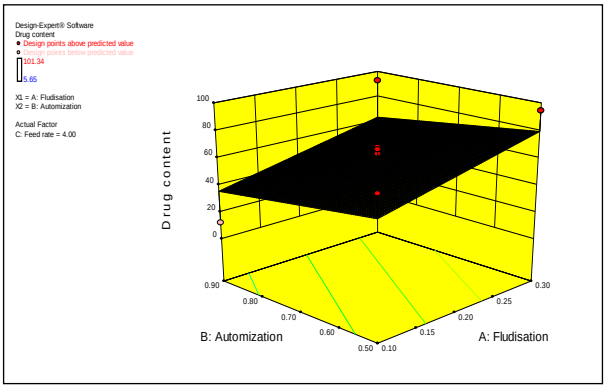

Figure 1: 3D response curve of drug content for drug layered pellets

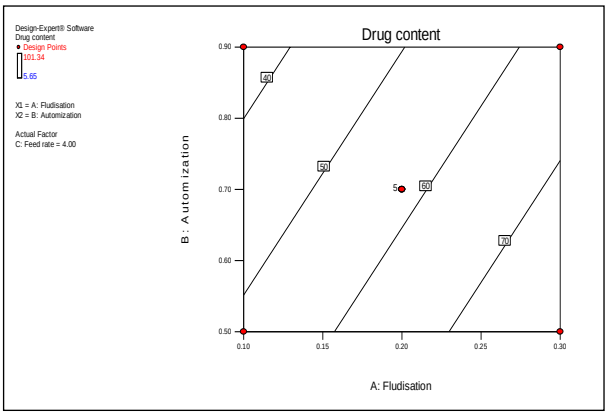

Figure 2: Contour plot for drug content for drug layered pellets

Table 15: Analysis of variance for response Y (Drug content).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Significance
Model	14427.93	3	4809.31	15.43	0.0001	S
X_1-Fludisation	*1526.01*	*1*	*1526.01*	*4.90*	*0.0454*	S
X_2-Automization	*520.84*	*1*	*520.84*	*1.67*	*0.2187*	NS
X_3-Feed rate	*12381.08*	*1*	*12381.08*	*39.72*	*< 0.0001*	S
Residual	4052.67	13	311.74			
Lack of Fit	*4037.04*	*9*	*448.56*	*114.82*	*0.0002*	
Pure Error	*15.63*	*4*	*3.91*			
Cor Total	18480.60	16				

***S – Significance #NS – Non-Significance**

Increase in fluidization pressure leads to even spreading of the drug solution and proper drying with minimum loss of drug. Increase in atomization pressure leads to reduction in droplet size of the coating solution. Therefore the solvent evaporation was fast before the drop reaches to the sugar spheres and therefore there is loss of coating solution. Whereas increase in feed rate and increase in atomization pressure with decrease in fluidization pressure leads to agglomeration and even sticking of the walls of Wurster process. Contour was plotted representing the maximum drug content versus fluidization and atomization. Here the maximum amount of drug decreases with increase in atomization and increases with increase in fluidization, reaching a maximum of a 70 mg/gm between 25±30 bar as shown in Fig 2. Optimization level of drug content 70 mg/gm at atomization (50-70 bar) and fluidization (25-30 bar).

Search for Optimum Processing Variables for Drug layering

The optimization design further gives the final optimized processing variables for drug layering and polymer coating. The optimized processing variables for drug layering was fluidization 0.16 bar, atomization 0.50 bar and feed rate 2 rpm. The final optimized processing variables were selected on the basis of the obtained results of its optimization trial. This selected batch was taken in triplicate. Drug content of drug layered pellets was given in table 16.The optimized processing variables of fluidized bed processor give the results within the limit. This indicates that the processing variables give the proper drug layering on sugar spheres.

Table 16: Drug content of Drug layered pellets

Selected Batch	Drug content (%)
1	99.84±2.3

Polymer coating

After design of trials for polymer coating all the trials was run. Then these trials were further evaluated for the weight gain and in vitro drug release from the polymer coated sugar spheres.

Dissolution Study

In vitro dissolution studies of Budesonide from pellets were performed in Gastric Fluid 1.2, Intestinal Fluid 6.8 and Phosphate buffer 7.4 using USP Type II dissolution test apparatus. *In vitro* release experiments were evaluated in order to investigate the release of drug from the polymer coated sugar spheres. Percentage cumulative release of drug coated pellets was shown in Table 17 and 18. The dissolution study of polymer coated sugar spheres releases drug at different rate. The obtained results of those trials were mention in the Table 19.

Table 17: Dissolution study of Polymer coated pellets (1-9)

Dissolution Fluid	Time (hr)	% Cumulative Drug Release								
		1	2	3	4	5	6	7	8	9
Gastric Fluid 1.2	1	47.92	1.09	1.86	79.23	83.44	1.36	27.67	80.02	37.47
	2	84.34	1.64	4.94	89.76	84.16	2.96	64.36		88.19
Intestinal Fluid 6.8	3		3.64	6.89			4.23	66.29		
	4		8.20	8.32			7.22	81.67		
	5		11.89	9.26			12.25			
Phosphate Buffer 7.4	6		37.72	16.36			33.21			
	7		54.72	23.12			49.59			
	8		71.63	29.40			61.32			
	9		80.15	35.02			75.36			
	10		88.54	46.37			80.23			
	11		88.28	53.35			80.63			
	12		89.37	59.32			81.21			

Table 18: Dissolution study of Polymer coated pellets (10-17)

Dissolution Fluid	Time (hr)	% Cumulative Drug Release						
		10	11	12	13	14	15	16
Gastric Fluid 1.2	1	81.13	1.09	1.09	1.09	1.09	1.900	0.28
	2		1.64	1.64	1.64	1.64	2.364	2.84
Intestinal Fluid 6.8	3		3.64	3.64	3.64	3.64	2.691	5.64
	4		8.20	8.20	8.20	8.20	3.915	9.34
	5		11.89	11.89	11.89	11.89	10.89	12.06
Phosphate Buffer 7.4	6		37.72	37.72	37.72	37.72	27.75	29.02
	7		54.72	54.72	54.72	54.72	35.53	46.67
	8		71.63	71.63	71.63	71.63	43.20	62.14
	9		80.15	80.15	80.15	80.15	68.65	71.11
	10		88.54	88.54	88.54	88.54	74.16	80.72
	11		88.28	88.28	88.28	88.28	74.39	81.08
	12		89.37	89.37	89.37	89.37	74.72	81.39

Table 19: Layout of design for polymer coating and its obtained response.

Run	Factor 1 Fluidization (bar)	Factor 2 Atomization (bar)	Factor 3 Feed Rate(rpm)	Response 1 Weight Gain (gm)
P 1	0.30	0.50	3.00	2.69±0.55
P 2	0.40	0.50	2.00	8.23±1.04
P 3	0.30	0.60	2.00	9.95±0.876
P 4	0.50	0.50	1.00	1.75±0.754
P 5	0.40	0.40	3.00	0.5±0.0965
P 6	0.50	0.60	2.00	9.1±0.987
P 7	0.30	0.40	2.00	3.83±1.005
P 8	0.40	0.40	1.00	0.37±0.005
P 9	0.50	0.50	3.00	2.11±0.54
P 10	0.50	0.40	2.00	0.2±0.05
P 11	0.40	0.50	2.00	8.23±1.43
P 12	0.40	0.50	2.00	8.23±1.03
P 13	0.40	0.50	2.00	8.23±0.913

P 14	0.40	0.50	2.00	8.23±0.713
P 15	0.40	0.60	3.00	9.47±1.10
P 16	0.30	0.50	1.00	9.25±0.94
P 17	0.40	0.60	1.00	9.38±1.10

With the increase in fluidization pressure than atomization pressure rate of heat transfer and mass transfer increases to such an extent that the drop as it reaches the nozzle tip is dried off and leads to clogging of nozzle. Similarly when fluidization pressure and atomization pressure were kept same the heat transfer and mass transfer was quite high that before the drop reached the drug loaded pellets it dried off. Due to which coating was impossible on drug loaded pellets. Whereas with increase in atomization pressure, decrease fluidization pressure with optimum feed rate gave proper coating on drug loaded pellets.

Table 20: Summary of results of regression analysis for responses Y_1 and Y_2.

Models	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response Y_1 quadratic model	0.9655	0.9212	0.4485	1.06	18.07
Response Y_2 quadratic model	0.9273	0.8339	-0.1627	17.27	35.68

Full and Reduced Model assessment for the dependent variables

The ranges of responses Y_1 and Y_2 was 0.2- 9.95 and 0 - 89.37% respectively. All the responses observed for seventeen processing variables were fitted to various models using Design- Expert software. It was observed that the best-fitted models were quadratic. The values of R^2, adjusted R^2, predicted R^2, SD and %CV are given in Table 20 along with the regression equation generated for each response. Summary of results of regression analysis for responses Y_1 and Y_2 was given in table 20.

Full Model equation

Weight Gain (Y_1)

Wt. Gain = +8.23-1.57 * X_1 +4.13 * X_2-0.75 * X_3

+0.69 * X_1* X_2+1.73 * X_1 * X_3-0.010

* X_2 * X_3-1.72 * X_1^2-0.74 * X_2^2-2.56 * X_3^2

It was observed that the independent variable viz. X_1 (Fluidization pressure) and X_3 (Feed Rate) had a negative effect on weight gain (Y_1). Another independent variable X_2 (Atomization Pressure) had a positive effect on weight gain (Y_1). The interaction of independent variables X_1 (Fluidization pressure), X_2 (Atomization Pressure) and X_1 (Fluidization pressure), X_3 (Feed Rate) had a positive effect on weight gain (Y_1). Interaction of X_2 (Atomization Pressure), X_3 (Feed Rate) had a negative effect on weight gain (Y_1). The quadratic effect of independent variables X_1 (Fluidization pressure), X_2 (Atomization Pressure) and X_3 (Feed Rate) had a negative effect on weight gain (Y_1).

Statistical validation of the polynomial equations generated by Design Expert and estimation of significance of the models was established on the basis of analysis of variance provision of the software as shown in Table 21.

Table 21: Analysis of variance for response Y_1 (Weight Gain).

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Significance
Model	220.39	9	24.49	21.79	0.0003	S
X_1Fluid. pres.	19.72	1	19.72	17.55	0.0041	S
X_2-Atom. Pres	136.13	1	136.13	121.12	< 0.0001	S
X_3-Feed Rate	4.47	1	4.47	3.98	0.0863	NS
X_1X_2	1.93	1	1.93	1.72	0.2312	NS
X_1X_3	11.97	1	11.97	10.65	0.0138	S
X_2X_3	4.000E-004	1	4.000E-004	3.559E-004	0.9855	NS
X_1^2	12.46	1	12.46	11.08	0.0126	S
X_2^2	2.31	1	2.31	2.05	0.1952	NS
X_3^2	27.59	1	27.59	24.55	0.0016	S
Residual	7.87	7	1.12			
Lack of Fit	7.87	3	2.62			
Pure Error	0.000	4	0.000			
Cor Total	228.25	16				

***S – Significant # NS – Non significant**

The Model F-value of 21.79 implies the model is significant. There is only a 0.03% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In Table 20, p values for response Y_1 (Weight Gain) represent that the quadratic contribution X_3, interaction X_1X_2, X_2X_3 and quadratic X_2^2 is non-significant model term. The quadratic contribution X_1, X_2 and interaction X_1X_3 and quadratic X_1^2, X_3^2 is significant model term. The equation indicates that fluidization pressure (X_1) has negative effect on the response Y_1 (weight gain). From the Figure 5 and 6 of the response curve of weight gain for polymer coated pellets, it is observed that as the fluidization pressure increases from -1 level (0.30 bar) to 0 (0.40 bar) and +1 level (0.50 bar), weight gain of polymer coated pellets decreases significantly. From equation indicate that atomization pressure (X_2) has positive effect on the response Y_1 (weight gain).On the other hand, the atomization pressure increases from -1 level (0.40 bar) to 0 level (0.50 bar) and +1 level (0.60 bar), weight gain of polymer coated pellets was increased. The equation indicates that feed rate (X_3) has negative effect on the response Y_1 (Weight gain). The feed rate increases from -1 level (1 RPM) to 0 level (2 RPM) and +1 level (3 RPM), weight gain of polymer coated pellets was decreased.

Contour was plotted representing the weight gain versus fluidization and atomization. Here the maximum weight gain decreases with increase in fluidization and increases with increase in atomization, reaching a maximum of a 10 gm between 55±60 bar as shown in fig 15. Optimization level of weight gain 10 gm at atomization (55-60 bar) and fluidization (35-40 bar).

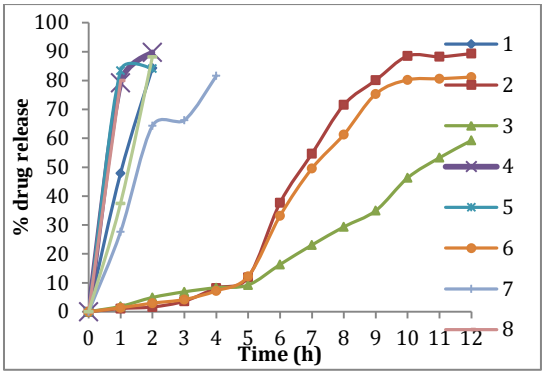

Fig. 3: Drug Release Study from the pellets coated with the Eudragit RLPO (1-9)

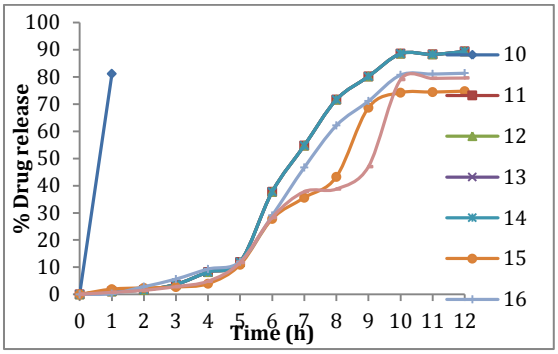

Fig. 4: Drug Release Study from the pellets coated with the Eudragit RLPO(10-17)

Percent Drug Release

$$\%\text{Drug Release} = +89.37 - 7.44 * X_1 + 36.86 * X_2 - 10.79 * X_3 + 5.47 * X_1 * X_2 + 20.35 * X_1 * X_3 - 1.22 * X_2 * X_3 - 36.24 * X_1^2 - 18.00 * X_2^2 - 32.79 * X_3^2$$

It was observed that the independent variable viz. X_1 (Fluidization pressure) and X_3 (Feed Rate) had a negative effect on Drug release (Y_2). Another independent variable X_2 (Atomization Pressure) had a positive effect on Drug release (Y_2). The interaction of independent variables X_1 (Fluidization pressure), X_2 (Atomization Pressure) and X_1 (Fluidization pressure), X_3 (Feed Rate) had a positive effect on Drug release (Y_2). Interaction of X_2 (Atomization Pressure), X_3 (Feed Rate) had a negative effect on Drug release (Y_2). The quadratic effect of independent variables X_1 (Fluidization pressure), X_2 (Atomization Pressure) and X_3 (Feed Rate) had a negative effect on Drug release (Y_2).Statistical validation of the polynomial equations generated by Design Expert and estimation of significance of the models was established on the basis of analysis of variance provision of the software as shown in Table 22. The Model F-value of 9.92 implies the model is significant. There is only a 0.31% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

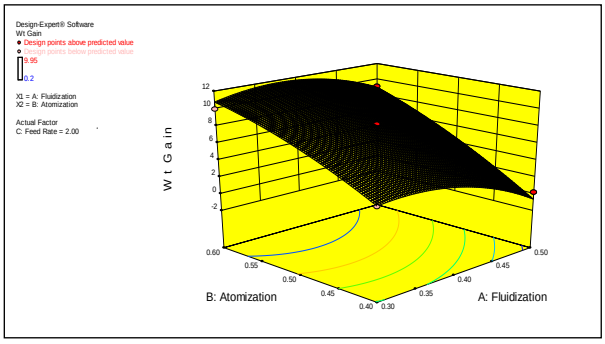

Figure 5: 3D response curve of weight gain for polymer coated pellets

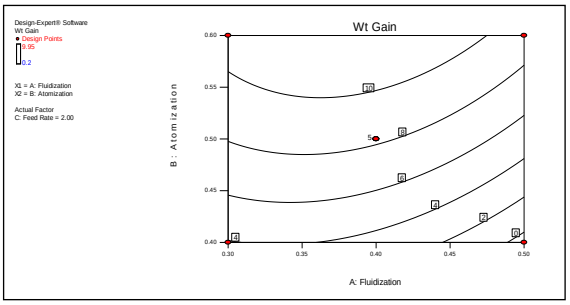

Figure 6: Contour plot of weight gain for polymer coated pellets

In Table 22, p values for response Y_2 (Drug Release) represent that the quadratic contribution X_1, X_3, interaction X_1X_2, X_1X_3, X_2X_3 and quadratic X_2^2 is non-significant model term. The quadratic contribution X_2 and quadratic X_1^2, X_3^2 is significant model term. The equation indicates that fluidization pressure (X_1) has negative effect on the response Y_2 (Drug Release). From the Figure 7 and 8 of the response curve of drug release for polymer coated pellets, it is observed that as the fluidization pressure increases from -1 level (0.30 bar) to 0 (0.40 bar) and +1 level (0.50 bar), drug release of polymer coated pellets decreases significantly. From equation indicate that atomization pressure (X_2) has positive effect on the response Y_2 (Drug Release).On the other hand, the atomization pressure increases from -1 level (0.40 bar) to 0 level (0.50 bar) and +1 level (0.60 bar), drug release of polymer coated pellets was increased. The equation indicates that feed rate (X_3) has negative effect on the response Y_2 (Drug Release). The feed rate increases from -1 level (1 RPM) to 0 level (2 RPM) and +1 level (3 RPM), drug release of polymer coated pellets was decreased.

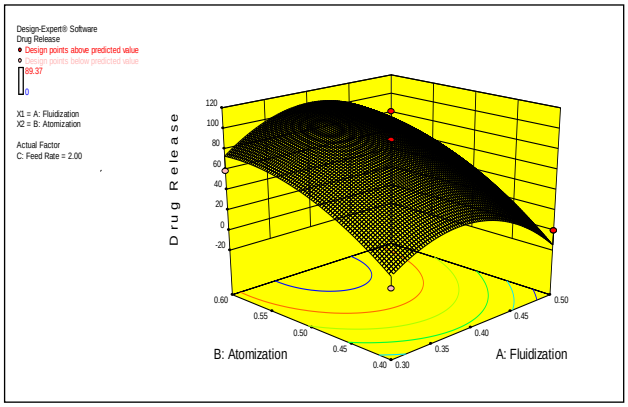

Figure 7: 3D response curve of drug release for polymer coated pellets

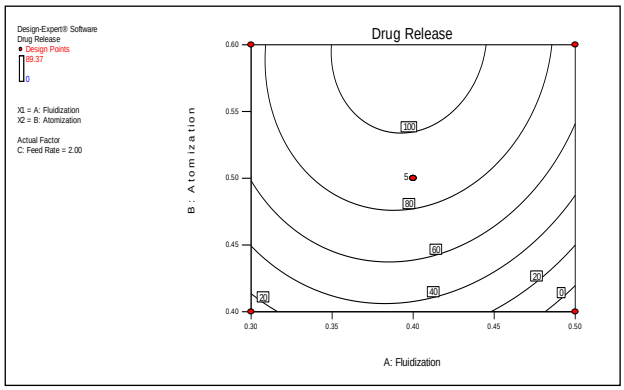

Figure 8: Contour plot of drug release for polymer coated pellets

Table 22: Analysis of variance for response Y_2 (Drug release).

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Significance
Model	26652.83	9	2961.43	9.92	0.0031	S
X_1-Fluidization	442.53	1	442.53	1.48	0.2627	NS
X_2-Atomization	10867.80	1	10867.80	36.42	0.0005	S
X_3-Feed Rate	930.53	1	930.53	3.12	0.1207	NS
X_1X_2	119.79	1	119.79	0.40	0.5465	NS
X_1X_3	1656.08	1	1656.08	5.55	0.0506	NS
X_2X_3	5.98	1	5.98	0.020	0.8914	NS
X_1^2	5528.70	1	5528.70	18.53	0.0035	S
X_2^2	1364.40	1	1364.40	4.57	0.0698	NS
X_3^2	4526.06	1	4526.06	15.17	0.0059	S
Residual	2088.68	7	298.38			
Lack of Fit	2088.68	3	696.23			
Pure Error	0.000	4	0.000			
Cor Total	28741.51	16				

***S – Significant # NS – Non significant**

Contour was plotted representing the drug release versus fluidization and atomization. Here the maximum drug release decreases with increase in fluidization and increases with increase in atomization, reaching a maximum of a 100% between 55±60 bar as shown in Fig 7. Optimization level of drug release 100% at atomization (55-60 bar) and fluidization (35-40 bar).

The optimization shows the two responses for the polymer coating that is weight gain and drug release. The weight gain was more means the polymer coating on the pellets was more. The polymer coating was more means the drug release from the pellets was decreases. From these observations the weight gain was inversely proportional to the drug release.

Search for Optimum Processing Variables for Polymer coating

The optimization of the processing variables was done on the basis of the results obtained in the above batches and required drug release. The optimized batch (P2) was having the processing variables fluidization pressure 0.40 bar, atomization pressure 0.50 bar and feed rate 2 rpm which showed a good desired release patterns and desirability 1.0. The optimized batch (P2) was identified to provide desired values for weight gain (8.24 gm) and percentage drug released at a 12 h (89.37%) (table 23 and 24).The optimized processing variables were selected. Then polymer coated batch was prepared and subjected to evaluation. The weight gain was determined for each batch. *In vitro* dissolution studies of Budesonide from pellets were performed in Gastric Fluid (1.2), Intestinal Fluid (6.8) & Phosphate Buffer (7.4) using USP Type I dissolution test apparatus. *In vitro* release experiments were evaluated in order to investigate the release of drug from the polymer coated pellets.

Table 23: Weight gain for polymer coated pellets of optimized batch p2

Sr. No.	Weight Gain
1	8.24±0.78

Table 24: Drug release of polymer coated pellets of optimized batch p2

Time	% Drug Release
1	1.12±0.17
2	1.94±0.21
3	4.64±1.06
4	9.27±0.99
5	12.89±1.11
6	40.12±4.28
7	57.72±1.95
8	69.63±2.03
9	82.15±1.61
10	87.54±0.5
11	88.28±1.16
12	89.25±0.98

Comparison of the SEM of the optimized sugar spheres

Appearance

The appearance of the sugar spheres shows that how they get coat. The plain sugar spheres were rough which is observed in the SEM. After drug layering the sugar spheres shows smooth appearance as compare to the plain spheres. Then on the drug layered the polymer coating was done which shows more smooth appearance as compare to the drug layered sugar spheres.

Size

Size of the spheres was increased on each coating. Initially the spheres size was 705 µm then on drug layering the size was 758 µm it shows the size on drug layering was increased. Then the polymer coating was done then size measured it shows that in size was 795 µm which is more than drug layered spheres (Figure 9,10,11).

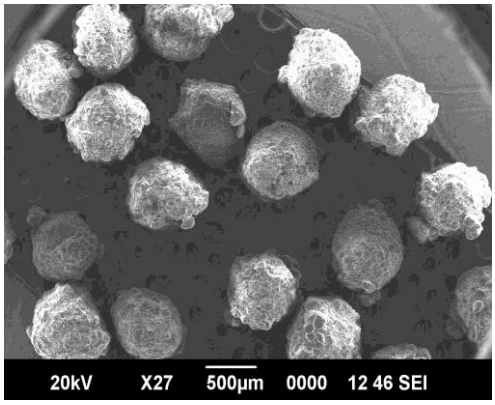

Figure 9: Plain Sugar sphere

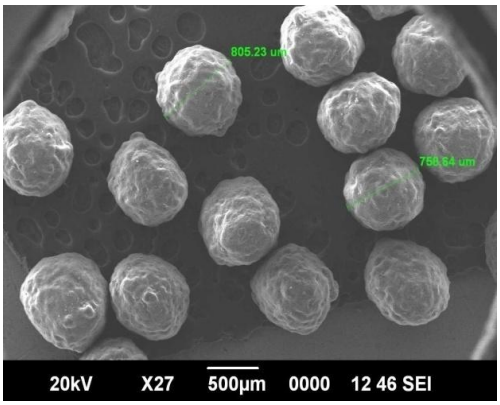

Figure 10: Drug (Budesonide) layered pellets

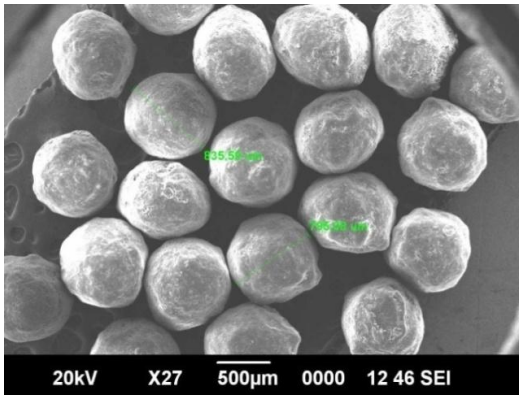

Figure 11: Eudragit RLPO coated pellets

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REFERENCES

1. Samvedna Aggarwal, Shalini Sharma, Sukhbir Lal, Neeraj Choudhary, Recent trends in Colon targeted drug delivery system, RJPBCS , 2(4), 2011;406-411.
2. Mayur M. Patel, Santnu L. Patel, Manish N. Bhadani, Tejal J. Shah and Avani F. Amin, A synchronous colon-specific drug delivery system for orally administered Mesalamine, Acta Pharmaceutica Sciencia,51: 251- 260 (2009).
3. M Chickpetty, Raga Baswaraj, B.K Nanjwade,studies on develop ment of novel combined time and pH-dependent solventless compression coated delivery systems for colonic deli very of
diclofenac sodium ,Asian Journal of Pharmaceutical and Clinical Research ,3(2), 2010, 410-416.
4. Shailendra Wasnik, Poonam Parmar, The design of colon-specific drug delivery system and different approaches to treat colon disease, International Journal of Pharmaceutical Sciences Review and Research , 6(2) , 2011; Article-031.
5. K.V. Vinay Kumar,T. Sivakumar, T. Tamizh mani, Colon targeting drug delivery system: A review on recent approaches, Int J Pharm Biomed Sci, 2(1), 2011; 11-19.
6. Krishnamachari Y, Madan P, Lin S. Development of pH- and time-dependent oral microparticles to opti- mize budesonide delivery to ileum and colon. Int J Pharm; 338(1-2): 2007; 238-47.
7. Postma DS, Kerstjens HAM. Are inhaled glucocorticosteroids effective in chronic obstructive pulmonary disease? Am J Respir Care Med.;160: 1999; 566-571.
8. Fedorak RN, Bistritz L. Targeted delivery, safety, and efficacy of oral enteric-coated formulations of budesonide. Adv Drug Deliv Rev; 57(2): 2005;303-16.
9. M.K. Chourasia, S.K. Jain, Pharmaceutical approaches to colon targeted drug delivery systems, Eur. J. Pharm. Sci. 6 (1); 2003; 32–66.
10. L. Yang, J.S. Chu, J.A. Fix, Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation, Int. J. Pharm. 235;2002; 1–15.
11. Karrout Y., Neut C., Wils D., Siepmann F., Deremaux L., Flament M.P., Dubreuil L., Desreumaux P., Siepmann J., Novel polymeric film coatings for colon targeting: Drug release from coated pellets, European Journal of Pharmaceutical Sciences 37,2009; 427–433.
12. Larsen C.C., Sonnergaard J.M., Bertelsen P., Holm P., A new process control strategy for aqueous film coating of pellets in fluidised bed, European Journal of Pharmaceutical Sciences 20, 2003; 273–283.
13. The united state pharmacopoeia. 29-NF. The official compendia of standards. Asian edition. United state pharmacopoeial convention, Inc., 2006; 2858, 2855
14. Akhgari, Garekani A H., Sadeghi F., Azimaie M., Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery, International Journal of Pharmaceutics 305,2005;22–30.
15. Annadurai G., Sheeja R.Y., Use of Box-Behnken design of experiments for the adsorption of verofix red using biopolymer, Bioprocess Engineering. 18,1998; 463-466.
16. Motwani S.K., Chopra S., Talegaonkar S., Kohli K., Ahmad F.J., Khar R.K., Chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and in vitro characterization, European Journal of Pharmaceutics and Biopharmaceutics, 53; 2007; 235-243.
17. Singh S.K., Dodge J., Durrani M.J., Khan M.A. Optimization and characterization of controlled release pellets coated with an experimental latex: I. Anionic drug, International Journal of Pharmaceutics. 125,1995;243-255.